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CASE REPORT

All-trans retinoic acid (ATRA) in non-promyelocytic acute myeloid leukemia (AML): results of combination of ATRA with low-dose Ara-C in three elderly patients with *NPM1*-mutated AML unfit for intensive chemotherapy and review of the literature

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Key Clinical Message

Based upon the clinical behavior of three patients, we suggest that the combination of low-dose Ara-C and all-trans retinoic acid may potentially be effective in some elderly patients, unfit for intensive chemotherapy, affected with *NPM1*-mutated acute myeloid leukemia without *FLT3* mutations, warranting perspective clinical studies in these selected patients.

Keywords

All-trans retinoic acid, elderly patients, low-dose Ara-C, *NPM1*-mutated acute myeloid leukemia, unfitness for intensive chemotherapy

Introduction

The addition of pharmacological doses of all-trans retinoic acid (ATRA) to chemotherapy has clearly revolutionized the clinical outcome of acute promyelocytic leukemia (APL) [1]. In vitro observations have also suggested that exposure to ATRA may sensitize both non-APL acute myeloid leukemia (AML) cell lines and primary cells to cytotoxic agents, such as Ara-C and anthracyclines, thereby increasing either differentiation or apoptosis, by

down-regulation of Bcl2 and related proteins [1, 2]. Based upon these preclinical data demonstrating a synergistic effect of ATRA combined with chemotherapy, several clinical studies, including large randomized trials (Table S1), have investigated the impact of adding ATRA to either remission induction chemotherapy or lower intensity regimens in patients with non-APL AML, but these studies have overall yielded inconsistent and conflicting results [1]. However, making direct comparisons between studies reporting discrepant clinical outcomes is difficult because of different patient age, leukemia characteristics, chemotherapy regimens and schedule of ATRA administration [1, 3–5].

Low-dose Ara-C (LDAC) has been widely used with different schedules within phase II trials for elderly patients with AML considered unfit for intensive chemotherapy, obtaining complete remission (CR) in about 20% of cases, but with unsatisfactory two-year overall survival (OS) rates of approximately 10% [2]. LDAC has become the prototype for low-intensity chemotherapy after the randomized trial by Burnett et al. in which LDAC produced higher CR rate (18% vs. 1%) and better OS, when compared to palliative hydroxyurea [2]. However, median OS (5 months) was still poor for the LDAC cohort and survival advantage was not recorded for patients with adverse cytogenetics, in whom CR was never achieved. Moreover, in this study, the addition of ATRA to either of the treatment arms had no beneficial effect on OS [2]. Conversely, some previous studies had shown positive effects of combining ATRA with LDAC, also in poor risk patients with AML unsuitable for aggressive chemotherapy [6, 7]. Venditti et al. reported a high CR rate (48%) in 33 patients with poor prognosis AML, either at disease onset or relapsed/refractory [6]. Moreover, in the retrospective study on 28 patients by Di Febo et al., a significant improvement in OS was observed, compared with LDAC alone, although the combination treatment did not increase the CR rate [7]. Unfortunately, none of these studies focused on the presence of *NPM1* gene mutations in leukemic cells [2, 6, 7]. Of note, retrospective molecular examinations on archival samples would potentially be of interest in order to precisely evaluate the clinical impact of ATRA combined with LDAC in specific molecular subgroups of unfit patients with AML [2, 6, 7].

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We report on three elderly patients affected with cytogenetically normal *NPM1*-mutated AML, without *FLT3* and *IDH1*-R132 mutations, considered unfit for intensive chemotherapy, because of advanced age and/or comorbidities (Table 1), who received moderate intensity treatment consisting of LDAC combined with ATRA. Patients have been hospitalized at AML diagnosis and received the first treatment course as inpatients. In details, LDAC 20 mg was administered subcutaneously twice daily on days 1–10, while ATRA 45 mg/m^2/day was given orally for 60 days (from day +3 to +62). Patients 1, 2, and 3 have spent 24, 22, and 20 days in the hospital, respectively, thereafter they were discharged and safely managed in an outpatient setting. Subsequent LDAC cycles have been administered, after intervals of 4 weeks, while each subsequent ATRA course has been started after 1 month interval (e.g., second ATRA course starting from day +3 of the fourth LDAC cycle). After two LDAC cycles combined with one ATRA course, morphologic CR was documented on bone marrow (BM) aspirate in patients 1 and 3, whereas in patient 2, BM aspirate was unfortunately not performed. However, in this latter patient, normal WBC and platelet counts, without circulating blasts, were obtained, with a concurrent reduction of RBC transfusion requirement. Unfortunately, on day +6 of the fourth LDAC cycle, disease progression was observed with WBC count 64.8 $\times$ 10^9/L and 70% circulating blasts, Hb 8.1 g/dL, Plt count 12 $\times$ 10^9/L. The patient died a few days later, 5 months since AML diagnosis. Patient 1 underwent nine LDAC cycles combined with three ATRA courses without experiencing any complication; then, treatment was withdrawn, while persisting morphologic CR. However, 9 months after therapy interruption, leukemia relapsed with circulating and BM blast counts 2% and 15%, respectively. Retreatment with the same previously administered cytotoxic regimen was attempted. The patient then received LDAC (four cycles) and ATRA (two courses) and 2 months after initiation of therapy, full hematologic recovery was documented, concurrently with a reduction in BM blast count (5–10%). However, the patient subsequently suffered from pneumonia, sinusitis and hematuria secondary to bladder mucosal lesions, with successive occurrence of pancytopenia, and died 8 and 26 months after relapse and first AML diagnosis, respectively. Patient 3 showed transient grade 2 gastrointestinal toxicity during first LDAC cycle, without any further relevant toxicity, until leukemia relapse was documented while receiving the tenth LDAC and third ATRA courses, respectively. He died a few weeks later, 11 months since initial diagnosis. Unfortunately, we have not measured quality of life (QoL) with validated instruments in these three patients.

Other AML patients, in particular with *NPM1* wild type, unfit for intensive chemotherapy, have not been treated with the same therapeutic strategy (LDAC + ATRA) at our Institution.

Discussion

Several experimental evidence supports the modulation of the retinoic acid-signaling pathway as a potential target

Table 1. Clinical characteristics of three elderly patients with *NPM1*-mutated AML not eligible for intensive chemotherapy.

Pt	Age (years)/Sex	Comorbidities	PS (ECOG score)	CBC at diagnosis (WBC/Plt counts x 10^9/L/Hb g/dL)	LDH level at diagnosis (IU/L)	PB/BM blasts at diagnosis (%)	Immunophenotype of myeloid blasts	Cytogenetics	*NPM1*/*FLT3*/*IDH1*-R132 mutational status on molecular examinations	Prognostic scores, according to Wheatley et al. [23]/Rollig et al. [20]	No. of cycles LDAC/ATRA	CR/DFS (months)/OS (months)
1	77/M	Prostatic carcinoma, peripheral neuropathy	1	1.5/121/11.4	293	2/40	CD34−, CD117−/+, CD33+, CD15+, CD13+, CD14−, CD64+, CD38+, DR−/+, CD11b+/−, CD66b+/−	Normal karyotype	Mutated/ WT/WT	Score 8, standard risk/Score 1, good intermediate risk	Nine cycles of LDAC, combined with three ATRA courses, then withdrawal. Subsequently, at relapse, four cycles of LDAC, combined with two ATRA courses..	Yes/16/26
2	72/F	Arterial hypertension, hypothyroidism, depression, dementia	3	6.2/60/10.6	455	27/40	CD34−, CD33+, CD13+, CD38+, DR−, CD11b−	Normal karyotype	Mutated (type A)/ WT/WT	Score 9, poor risk/Score 1, good intermediate risk	Three cycles of LDAC, combined with one ATRA course	NA (HI)/NA/5
3	79/M	Arterial hypertension, benign prostatic hyperplasia	1	46.9/255/10.7	373	34/60	CD34−, CD33+, CD13+, CD15+, CD14+, CD64+, CD11a+, cMPO+, DR+, CD56+/−, CD11b+	Normal karyotype	Mutated (type A)/ WT/WT	Score 9, poor risk/Score 3, good intermediate risk	Ten cycles of LDAC, combined with three ATRA courses	Yes/7/11

NPM1, nucleophosmin; AML, acute myeloid leukemia; Pt, patient; ys, years; PS, performance status; ECOG, Eastern Cooperative Oncology Group; CBC, complete blood count; WBC, white blood cell; Plt, platelet; Hb, hemoglobin; LDH, lactate dehydrogenase; PB, peripheral blood; BM, bone marrow; *FLT3*, FMS-like tyrosine kinase 3; *IDH1*, isocitrate dehydrogenase 1; WT, wild type; LDAC, low-dose Ara-C; ATRA, all-trans retinoic acid; CR, complete remission; DFS, disease-free survival; OS, overall survival; HI, hematologic improvement; NA, not applicable.
Unfitness for intensive chemotherapy was defined according to Ferrara et al., Leukemia 2013 [25].

for therapy in *NPM1*-mutated AML [8–12]. Earlier in vitro studies by Martelli et al. showed that pharmacological doses of ATRA induced cell cycle arrest and apoptosis in both *NPM1*-mutated cell line OCI-AML3 and primary leukemic cells propagated in NOD-SCID mice, by selectively down-regulating the NPM1 mutant protein at post-transcriptional level [8]. Moreover, Kutny et al. demonstrated that *NPM1*-mutated AML cells may also be susceptible, in vitro, to the pro-differentiating properties of ATRA [9]. Further in vitro findings also suggested that targeted depletion of NPM1 protein may selectively sensitize *NPM1*-mutated AML cells to Ara-C and ATRA [10]. Of note, it has recently been reported that exposure of *NPM1*-mutated AML cells to ATRA and arsenic trioxide (ATO) induces selective proteasome-mediated degradation of NPM1 mutant protein accompanied by nucleolar redistribution of wild-type NPM1, reversal of the characteristic disorganization of PML bodies and pronounced apoptosis and/or differentiation [11, 12]. Strikingly, NPM1 mutant protein down-regulation by ATRA/ATO was shown to potentiate response to daunorubicin [11].

Available information on the clinical use of ATRA in adjunct to other antileukemic treatments in *NPM1*-mutated patients with AML is summarized in Table 2 [3–5, 12–18]. Interestingly, in a retrospective biomarker analysis within the randomized HD98B trial, Schlenk et al. showed that the addition of ATRA to conventional chemotherapy, including etoposide, significantly improved event-free survival and OS only in the subgroup of elderly patients with *NPM1*-mutated AML without *FLT3*-ITD mutation [3]. Furthermore, preliminary data from the prospective randomized treatment trial AMLSG 07-04 for younger patients with AML seem to confirm the results obtained in elderly patients [18]. In details, the beneficial effect of ATRA on OS in the whole cohort could be attributed to patients with favorable risk AML, including *NPM1*-mutated AML in the absence of *FLT3*-ITD [18]. Intriguingly, biological observations from these series suggested that repressor activity on retinoic acid signaling induced by high-PRAME levels may be overcome by the addition of ATRA [19]. Conversely, the randomized MRC AML12 trial for patients AML <60 years of age did not identify any molecular subgroup, defined by mutations in *NPM1, FLT3, CEBPA* genes, likely to derive a significant survival benefit from the addition of ATRA to aggressive chemotherapy [4]. Consistently, in an analysis stratified by etoposide addition and *NPM1/FLT3* mutational status, there was no significant improvement in clinical outcomes by the addition of ATRA to intensive chemotherapy for any subgroup of older patients enrolled in NCRI AML16 trial [15]. Moreover, Nazha et al. observed that the addition of ATRA to chemotherapy did not affect overall outcome of patients with AML regardless of *NPM1* mutational status [5]. However, it should be noted that limitations of this study include the small sample size (20 *NPM1*-mutated patients with AML) and that other gene mutations, such as *FLT3*-ITD, have not been investigated in this retrospective analysis [5]. Overall, there is currently no consensus as to whether the addition of ATRA to chemotherapy improves the clinical outcome of *NPM1*-mutated patients with AML (Table 2) [1, 3–5, 15, 18]. Of note, dosing schedule and timing of ATRA administration, namely before, simultaneously or after exposure to conventional chemotherapy, may be relevant to explain discrepancies observed in different series [3–5, 15, 18]. Of interest, in vitro experiments indicated that synergistic effects on cell viability were only observed when ATRA was given after exposure to cytotoxic drugs [3]. Indeed, in our three patients, such as in Austrian-German trials, ATRA administration has been started a few days after chemotherapy, a timepoint when leukemic cells have already been exposed to a significant cytotoxic effect [3, 18]. However, in addition to the potential efficacy of LDAC in association with ATRA, we acknowledge that the relatively favorable clinical outcome observed in two of our three elderly patients with AML, may also have been attributed to the less aggressive molecular features, namely *NPM1* gene mutation without *FLT3* gene mutations, documented in leukemic cells [20–23].

Despite the huge amount of data, although conflicting, regarding *NPM1*-mutated patients with AML treated with ATRA combined with intensive chemotherapy, scanty information is so far available on the association of ATRA and LDAC in this molecular subgroup of patients, when elderly and fragile [14]. Based upon our clinical observations, we suggest that the combination of LDAC and ATRA may potentially be effective in some elderly patients, unfit for intensive chemotherapy, affected with *NPM1*-mutated AML without *FLT3* mutations, a relatively good prognosis AML. Although *IDH1*-R132 mutation has not been documented in our patients, it should be noted that ATRA at clinically achievable doses has recently been shown to markedly enhance terminal granulocytic differentiation in vitro, in either AML cell lines or primary patient samples carrying mutant *IDH1* [24]. Moreover, a potent antileukemic effect of ATRA was observed in the presence of *IDH1*-R132H mutation in a xenograft mouse model, suggesting that *IDH1*-R132 mutation could be a valuable biomarker to select patients with AML for ATRA treatment [24].

Perspective randomized clinical trials are warranted to compare LDAC alone versus LDAC combined with ATRA and/or ATO, in order to clarify the exact role of such treatments in these selected genetic subsets of fragile patients [25]. In addition to the assessment of the efficacy in terms of response rates and survival, further endpoints,

Table 2. Clinical use of ATRA in patient with *NPM1*-mutated AML: review of the literature.

Reference	Number of patients/clinical characteristics	Treatment schedule	Outcome	Comments
Hutter et al., 2008 [13]	A total of 171 elderly patients with *NPM1*-mutated AML enrolled in two consecutive AMLSG protocols and included in a retrospective analysis.	Seventy-eight patients (median age 67.8 years) from trial A, AML HD98B. Ninety-three patients (median age 67.9 years) from trial B, AMLSG 06-04, in which idarubicin was intensified in induction therapy and etoposide was omitted. 37% and 94% of patients received ATRA in trials A and B, respectively.	CR 68% and 71% in trials A and B, respectively. No significant difference in OS between the two cohorts. Restricting the analysis to patients who received ATRA, better EFS and DFS for *NPM1*-mutated/*FLT3*-ITD negative patients in trial A compared to trial B.	Etoposide in combination with ATRA may exert a beneficial synergistic effect in elderly patients with AML having *NPM1* mutation without concurrent *FLT3*-ITD.
Schlenk et al., 2009 [3]	A total of 377 patients with de novo or secondary AML, enrolled into the randomized AMLSG HD98B treatment trial. Median age 67 years (range 61–84). *NPM1* mutations present in 60 of the 254 analyzed samples (24%).	Two induction cycles with idarubicin, standard-dose cytarabine and etoposide with or without ATRA (45 mg/m^2 on days 3–5 and then 15 mg/m^2 on days 6–28), followed by one consolidation cycle of intermediate-dose cytarabine and mitoxantrone with or without ATRA (15 mg/m^2 on days 6–28). For second consolidation, patients were randomized to either intensive therapy with idarubicin and etoposide or oral maintenance therapy.	Patients randomized to ATRA had significantly better RFS and OS, with 4-years RFS and OS rates 20.9% and 10.8%, respectively, as compared to 4.8% and 5%, respectively, in the standard treatment arm.	A significant interaction between *NPM1*-mutated AML without *FLT3*-ITD and treatment with ATRA was identified, in that the beneficial effect of ATRA on RFS and OS was restricted to this subgroup of patients.
Burnett et al., 2010 [4]	A total of 1075 adult patients with AML, enrolled in MRC AML12 randomized protocol. Median age 48 years (range 14–68). *FLT3*-ITD mutations were present in 137 (23%) and *NPM1* mutations in 207 (35%) of the 592 patients with available molecular data. Patients with *NPM1* and *FLT3* mutations were equally distributed between treatment groups.	Randomization in induction to two courses of daunorubicin 50 mg/m^2 on days 1,3,5, thioguanine 100 mg/m^2 every 12 h on day 10 in course 1 and on day 8 in course 2, cytarabine at a dose of either 100 mg/m^2 (standard DAT) or 200 mg/m^2 (high DAT) every 12 h on days 1–10 in course 1 and days 1–8 in course 2, each with or without ATRA 45 mg/m^2/day on days 1–60. Subsequently, patients received consolidation with course 3 (amsacrine, cytarabine, etoposide) and were randomized between one or two further courses, and to chemotherapy versus transplant.	Overall, there was no effect from the addition of ATRA (CR + CRi 83% with vs. 84% without ATRA; 8-year OS 33% with vs. 30% without ATRA).	The effect of ATRA was not significantly different in any of the four subgroups defined by the combination of *FLT3* and *NPM1* status. In *NPM1*-mutated AML without *FLT3*-ITD patients eight-year OS was 56% with ATRA and 40% without ATRA, but the difference was not statistically significant. There was a suggestion that ATRA reduced relapse in patients with lower MN1 levels, but no significant effect on OS was observed. This study did not identify any subgroup of patients likely to derive a significant survival benefit from the addition of ATRA.

(*Continued*)

Table 2. Continued.

Reference	Number of patients/clinical characteristics	Treatment schedule	Outcome	Comments
Fredly et al., 2013 [14]	Thirty-six patients with either previously untreated (de novo or secondary) or relapsed AML, unfit for conventional intensive chemotherapy. Median age 77 years (range 48–90). *NPM1* and *FLT3*-ITD mutations documented in 35% (13) and 40% (14) of the cases, respectively.	On day 1, initial intravenous loading dose of VPA, then oral therapy 300 mg twice daily, continued indefinitely to maintain therapeutic concentrations. ATRA 21.5 mg/m^2 twice daily on days 8–22 and repeated every 12th week. LDAC 10 mg/m^2/day on days 15–24 and then repeated every 12th week.	Overall, 11 of 36 patients showed response to treatment (2 CR, 9 HI). The most common response was increased and stabilized platelet counts. Median survival 171 days and 33 days in responders and nonresponders, respectively. Detailed clinical outcome of *NPM1*-mutated patients with AML is not reported.	Disease stabilization was seen in a subset of patients with AML. No significant differences with regard to age, gender, PB counts, de novo versus secondary AML, cytogenetic or molecular (*FLT3, NPM1*) abnormalities between responders and nonresponders.
Nazha et al., 2013 [5]	Seventy patients with NK-AML who were enrolled in a previous phase II randomized clinical trial and had stored BM samples for *NPM1* mutation analysis. Twenty (29%) patients had *NPM1* mutation. Among them, seven patients received ATRA + chemotherapy.	Patients were randomly assigned to receive, as remission induction treatment: (a) FAI regimen (fludarabine 30 mg/m^2 on days 1–4, Ara-C 2 g/m^2 on days 1–4, idarubicin 12 mg/m^2 on days 2–4); (b) FAI + G-CSF; (c) FAI + ATRA (45 mg/m^2/day); (d) FAI + ATRA + G-CSF. If WBC count was $<10 \times 10^9$/L, ATRA was begun 2 days before chemotherapy. If WBC count was $\geq 10 \times 10^9$/L, ATRA was begun on day 1. ATRA administration was continued for 3 days after completion of chemotherapy.	CR rate in patients with *NPM1* mutation was 71% and 69%, with or without ATRA, respectively. Median OS, EFS, RFS for the entire group were 11.5, 7, and 11.5 months, respectively.	The addition of ATRA to induction chemotherapy did not affect CR rate, OS, EFS, and RFS of patients with NK and *NPM1* mutation.
Burnett et al., 2013 [15]	A total of 616 older patients with either de novo or secondary AML or high-risk MDS, enrolled in the NCRI AML16 trial. Median age 67 years (range 53–82). *FLT3*-ITD and *NPM1* data available for 422 and 404 patients, with mutation rates of 19% and 24%, respectively.	Randomization to DA versus ADE and ATRA versus no ATRA in a 2 × 2 factorial design. Daunorubicin 50 mg/m^2 on days 1–3 and cytarabine 100 mg/m^2 every 12 h on days 1–10 (course 1) or days 1–8 (course 2). Patients allocated to ATRA arms, received ATRA 45 mg/m^2/day for 60 days. Etoposide in ADE arm was given at 100 mg/m^2 on days 1–5 of courses 1 and 2.	ORR 69% and two-year survival 35%. ORR not different between DA and ADE, although CR rates were nonsignificantly lower in patients given ATRA. At two-years, neither OR nor RFS differed between arms (OS: ADE 33% vs. DA 36%; ATRA vs. not 35% vs. 35%).	In an analysis stratified by etoposide and by *NPM1*/*FLT3* risk group, there was no significant heterogeneity of the effect of ATRA. No beneficial effect of ATRA in *NPM1*-mutated AML without *FLT3*-ITD appeared for patients receiving ADE.
Tassara et al., 2014 [16]	A total of 195 elderly (range 61–83 years) patients with either de novo or secondary AML. *NPM1* mutations were found in 22 and 18 of the	Randomization to receive induction either with (VPA group) or without (standard group) VPA. Induction therapy consisted of two cycles of idarubicin 12 mg/	CR rates after induction tended to be lower in VPA group (40%) compared with standard group (52%), as a result of the higher early death rate. After a	The addition of VPA to intensive induction chemotherapy and ATRA did not result in an improvement of CR rates, EFS and OS, mainly as a

(*Continued*)

Table 2. Continued.

Reference	Number of patients/clinical characteristics	Treatment schedule	Outcome	Comments
	available samples from patients in standard group (26%) and VPA group (22.5%), respectively	m^2 on days 1–3, cytarabine 100 mg/m^2 on days 1–5, ATRA 45 mg/m^2 on days 3–5 and 15 mg/m^2 day 6–28 (AIC) or by the same chemotherapy plus VPA 400 mg twice daily (V-AIC). After interim analyses, idarubicin was reduced to days 1 and 3, and VPA was given only during the first cycle. A second amendment stopped randomization because of toxicity and inferior CR rates in V-AIC arm. All patients in CR received two consolidation cycles with chemotherapy and ATRA.	median follow-up of 84 months, five-year EFS (2.3% in standard and 7.6% in VPA) and OS (11.7% in standard and 11.4% in VPA) were not different between the two groups. However, five-year RFS was significantly superior in VPA group (24.4%) compared with standard (6.4%).	result of increased VPA-related hematologic toxicity and higher death rates during second induction cycle. Explorative subset analyses revealed that *NPM1*-mutated AML may particularly benefit from VPA.
Guenounou et, 2014 [17]	Three patients, aged 16, 21 and 51 years, respectively, affected with relapsed/refractory *NPM1*-mutated with concurrent *FLT3*-ITD positivity.	Sorafenib (400 mg twice a day) and ATRA (45 mg/m^2/day on days 1–14). Each cycle was repeated every 28 days until progression or toxicity. Two patients received etoposide 150 mg/m^2 for 2 days for debulking.	Patient 1 obtained fourth CR; sorafenib was stopped after 2 years for toxicity and relapse occurred. Patient 2 was still in CR after 18 months of treatment (ATRA was stopped after 11 months for liver toxicity). Patient 3 received therapy bridge to transplant, without obtaining remission.	Patients with *FLT3*-ITD+ and *NPM1*-mutated AML could obtain unexpected responses upon treatment with the combination of sorafenib and ATRA, which could not have been achieved with conventional therapies (patients 1 and 2 were previously allografted).
Schlenk et al., 2014 [18]	A total of 1100 adult (age 18–60 years) with AML, entered in prospective randomized treatment trial AMLSG 07-04. *NPM1* mutation was documented in 29% of patients.	Induction therapy consisted of two cycles ICE (idarubicin 12 mg/m^2 on days 1,3,5 or on days 1,3 in cycle 2; cytarabine 100 mg/m^2 on days 1–7; etoposide 100 mg/m^2 on days 1–3). For consolidation therapy, high-risk patients received allo-HSCT, while all other patients were assigned to high-dose cytarabine (18 g/m^2 per cycle). Patients were randomized to receive ATRA (during induction 45 mg/m^2 on days 6–8, 15 mg/m^2 on days 9–21; during consolidation 15 mg/m^2 on days 6–28).	A PP analysis revealed higher probability for *NPM1*-mutated AML patients treated with ATRA to achieve a CR, with longer EFS. Explorative analysis in all patients on OS revealed a benefit for patients treated with ATRA compared to those who have not received ATRA (ITT, $P = 0.09$; PP, $P = 0.01$).	The beneficial effect of ATRA on OS in the whole cohort of younger patients could be attributed to patients with ELN-favorable risk including core-binding factor AML, AML with *CEBPA*dm and *NPM1*-mutated AML in the absence of *FLT3*-ITD.
El Hajj et al., 2015 [12]	Five elderly patients with previously untreated or relapsed *NPM1*-mutated AML, unfit for chemotherapy.	Compassionate use of ATRA 45 mg/m^2/day combined with ATO 0.1 mg/kg/day.	BM blasts significantly decreased in three patients and then re-increased upon treatment discontinuation. One patient died from IA at	Although CRs were not observed, ATRA + ATO exerted a transient in vivo antileukemic effect, with leukemia regression in some

(*Continued*)

Table 2. Continued.

Reference	Number of patients/clinical characteristics	Treatment schedule	Outcome	Comments
			day +21 with no evidence of response. Another patient rapidly died from bilateral pneumonia at day +10.	patients. The combination is unlikely to be curative alone, but may be part of a broader therapeutic strategy.

ATRA, all-trans retinoic acid; AML, acute myeloid leukemia; CR, complete remission; BM, bone marrow; G-CSF, granulocyte-colony stimulating factor; WBC, white blood cell; EFS, event-free survival; OS, overall survival; DFS, disease-free survival; MDS; myelodysplastic syndrome; VPA, valproic acid; CRi, complete remission with incomplete blood count recovery; ORR, overall response rate; ORR, overall response rate; PB, peripheral blood; RFS, relapse-free survival; NK, normal karyotype; HSCT, hematopoietic stem cell transplant; PP, per protocol; ITT, intention to treat; ELN, European LeukemiaNet; ATO, arsenic trioxide; IA, invasive aspergillosis.

such as transfusion requirements, achievement of transfusion independence, number and duration of hospitalizations per patient year, are actually recognized as extremely relevant, especially in clinical trials investigating moderate intensity treatments [26]. Moreover, QoL parameters and other patient-reported outcomes, assessed with validated instruments, should increasingly be incorporated as secondary endpoints in clinical studies for patients with AML [27].

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Conflict of Interest

BF applied for a patent on the clinical use of NPM1 mutants. The other authors report no potential conflicts of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Clinical use of ATRA in non-APL AML patients: review of the literature.